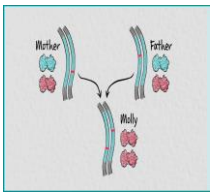


Genes as Medicine

hhmi | BioInteractive

Film Guide
Educator Materials

OVERVIEW

Gene therapy—the delivery of corrective genes into cells to treat a genetic disease—is an idea that was on scientists' minds as early as the 1960s. It took more than 35 years, however, to accumulate the knowledge and tools necessary to make gene therapy in humans a success. The HHMI short film *Genes as Medicine* tells the story of Drs. Jean Bennett, Albert Maguire, and their colleagues' decades-long effort to develop a gene therapy for a childhood disease called Leber congenital amaurosis (LCA).

KEY CONCEPTS

A. Some inherited diseases are caused by mutations in single genes. These mutations result in proteins that malfunction or, in some cases, no protein being produced, which cause the disease phenotypes.
B. For an individual to have a recessive genetic disease, they must have a disease-causing mutation in each copy (or allele) of a gene. If an individual has one allele with the mutation and one allele without it (in other words, they are heterozygous), they may have no disease symptoms.
C. Gene therapy is an experimental technique that adds corrective copies of mutated genes to a patient's cells.
D. Many biotechnology applications take advantage of naturally occurring processes. For example, in developing some gene therapies, scientists take advantage of viruses' ability to add genes to cells.
E. Most medical discoveries, including gene therapy, can take decades to move from the lab to new treatments available to people who need them.
F. Before most new medical treatments can be made available to patients, they must first be shown to work in animal models and then be shown to work in people taking part in clinical trials.

CURRICULUM CONNECTIONS

Standards	Curriculum Connections
NGSS (2013)	LS1.A, LS3.A, LS3.B
AP Biology (2015)	3.A.1, 3.C.1, 3.D.4, SP6
IB Biology (2016)	3.4, 3.5, B.4
Common Core (2010)	ELA.RST.9-12.2, WHST.9-12.4
Vision and Change (2009)	CC2, CC3

PRIOR KNOWLEDGE

Students should

- be familiar with the basics of gene expression, that genes code for proteins and proteins produce traits. If students need to review the process of gene expression, you may want to point them to the HHMI BioInteractive interactive "Central Dogma and Genetic Medicine" (http://www.hhmi.org/biointeractive/central-dogma-and-genetic-medicine).
- understand that for children to have a recessive genetic disease, they must have two copies of the gene (or two alleles) with disease-causing mutations.

PAUSE POINTS

The film may be viewed in its entirety or paused at specific points to review content with students. The table below lists suggested pause points, indicating the beginning and ending times in minutes in the film.

	Begin	End	Content Description	Review Questions
1	0:00	3:08	• Some inherited diseases are caused by genetic mutations that result in faulty or missing proteins. • Gene therapy treats inherited diseases by delivering corrective copies of mutated genes to affected tissues so that cells can produce functional proteins.	• Molly's form of blindness is inherited. What does that mean? • How could delivering new genes to cells change an organism's traits?
2	3:09	6:02	• The retina is a specialized neural tissue at the back of the eye that converts light energy into signals to the brain. • In gene therapy, viruses are used to deliver corrected genes to cells. Viruses can pass through membranes and deliver genetic information to cells. • Viruses used in gene therapy are modified to remove harmful genes and genes needed for viruses to multiply. These modified viruses contain the corrective gene and regulatory sequences that allow the gene to be expressed in the target cells.	• Dr. Maguire says that the retina is like "camera film." What does he mean? • What makes the eyes a good potential target for gene therapy? • Why are viruses used in gene therapy? • How do scientists modify viruses so that they can be used in gene therapy?
3	6:03	8:46	• Animal models are used to test the safety and efficacy of treatments before they are tested in humans. • Leber amaurosis is a form of inherited blindness. Some forms of the disease are caused by mutations to the *RPE65* gene. • Disease-causing mutations result in malfunctioning or missing RPE65 protein, which in turn causes photoreceptors to malfunction and eventually die. • Some Briard shepherds have Leber amaurosis caused by mutations in their *RPE65* gene. • Two mutated alleles of the *RPE65* gene are required for an individual to have the disease.	• Why are dogs a better animal model than mice for testing gene therapy treatments of Leber amaurosis? • What are photoreceptors and how are they affected in Leber amaurosis? • What does it mean that a disease is progressive? What makes Leber amaurosis a progressive disease? • Why didn't Leber amaurosis affect Molly's parents if they each had a mutated copy of *RPE65*? • Based on your previous knowledge, what type of disease is Leber amaurosis: X-linked, autosomal dominant, or autosomal recessive?
4	8:47	13:10	• Jesse Gelsinger, an 18-year-old boy with a rare liver disease, died shortly after participating in a gene therapy trial in 1999. • At around the same time, Drs. Jean Bennett and Albert Maguire showed that *RPE65* gene therapy restored sight in Briard shepherds with Leber amaurosis. • Because of this success, Dr. Bennett was asked to help design a gene therapy trial in human patients.	• Why did drug companies lose interest in gene therapy? • What do you think happened, at the molecular level, to the Briard shepherds treated with gene therapy? • Why did Dr. Katherine High ask Dr. Jean Bennett to work with her on a human trial?

5	13:11	17:16	• After years of work, Drs. Bennett, Maguire, and High successfully treated patients with Leber amaurosis in 2007. • After 40 patients, many of them children, were successfully treated, a panel of advisors to the Food and Drug Administration (FDA) recommended that the therapy be approved for use in humans.	• Why didn't the surgeons inject both of Molly's eyes with the treatment at the same time? • What benefit did Molly receive from participating in the gene therapy trial? • How does the development of gene therapy for Leber amaurosis demonstrate the importance of collaboration in science? • About how many years was Dr. Bennett working on this problem? Why did it take so long?

BACKGROUND

Gene therapy is a method for treating inherited diseases in which corrective versions of genes are delivered to cells that express defective versions of those genes. The development of this technology for use in patients has been on a roller coaster of advances and setbacks over the last two decades. Few scientists are more familiar with that roller coaster than Drs. Bennett and Maguire. They have focused their lives on trying to use gene therapy to treat an inherited form of blindness called Leber congenital amaurosis (LCA, also called "Leber amaurosis").

LEBER AMAUROSIS

LCA is one of the most common causes of inherited childhood blindness. It occurs in 2 to 3 out of 100,000 newborns and can result from mutations in one of at least 17 genes. We learn in *Genes as Medicine* that LCA can be caused by mutations in a gene called *RPE65* that is expressed in cells of the retina, specifically within the retinal pigment epithelium (RPE). The RPE is a layer of pigmented cells within the retina that wrap around the vertebrate eye just outside the photoreceptors (Figure 1).

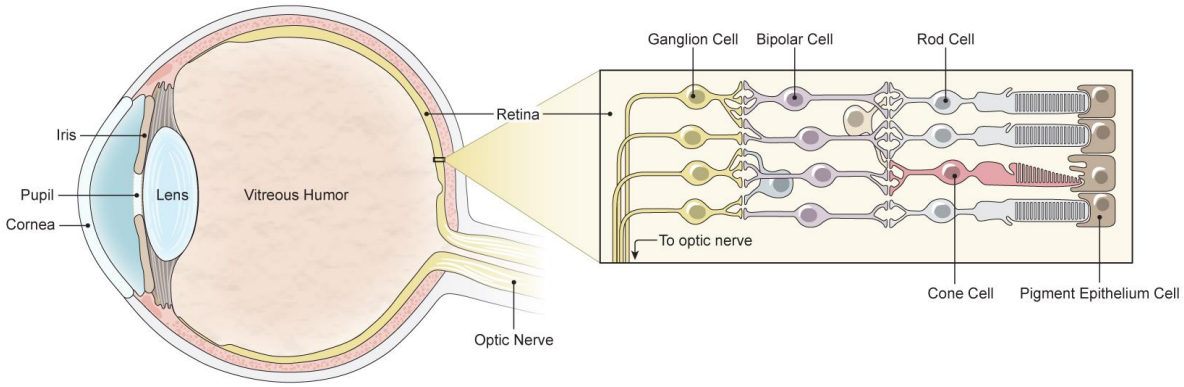


Figure 1. The retina is a layer of neuronal tissue at the back of the eye. Within the retina, the retinal pigment epithelium (RPE) cells provide proteins and other molecules essential to the function of the photoreceptor cells. Photoreceptors are made up of rods (light-detecting cells) and cones (color-detecting cells). In patients with LCA, the rods become damaged and die.

Although LCA can be caused by mutations to one of many different genes, the disease almost always follows an autosomal recessive pattern of inheritance. This means that to have LCA-induced blindness, an individual has to have inherited two copies of a mutated gene, one from each parent.

Mutations in *RPE65* (which stands for retinal pigment epithelium-specific 65kDa) were first associated with LCA in 1997. More than 30 different mutations in the *RPE65* gene have been identified to cause LCA. In the film, we learn that Molly inherited a mutated allele of *RPE65* from each of her parents. Her parents are therefore each carriers of LCA but are unaffected by any symptoms because they each have one allele that produces a functional RPE65 protein (Figure 2).

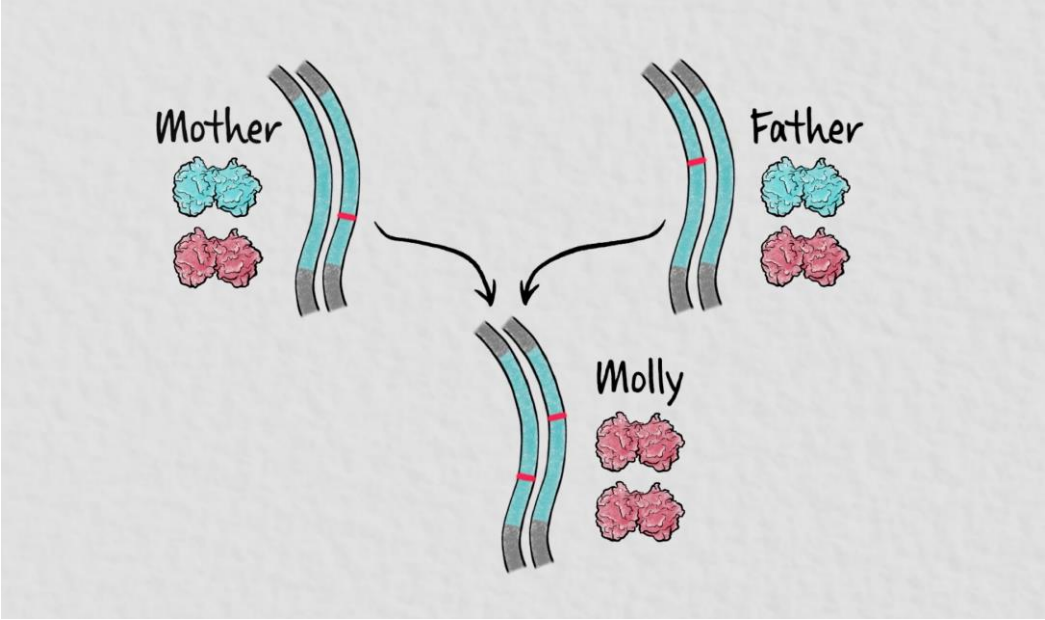


Figure 2. Molly has LCA because she inherited a mutant *RPE65* allele from each of her parents. The two mutations (represented by the red line) are different, but both result in a nonfunctional protein (shown as red instead of blue) being produced. Geneticists refer to someone with two different disease-associated alleles of the same gene as a compound heterozygote.

RPE65

The *RPE65* gene normally produces an enzyme (the RPE65 protein) that is involved in the visual cycle: a multistep process that converts light entering the eye into electrical signals transmitted to the brain (Figure 3).

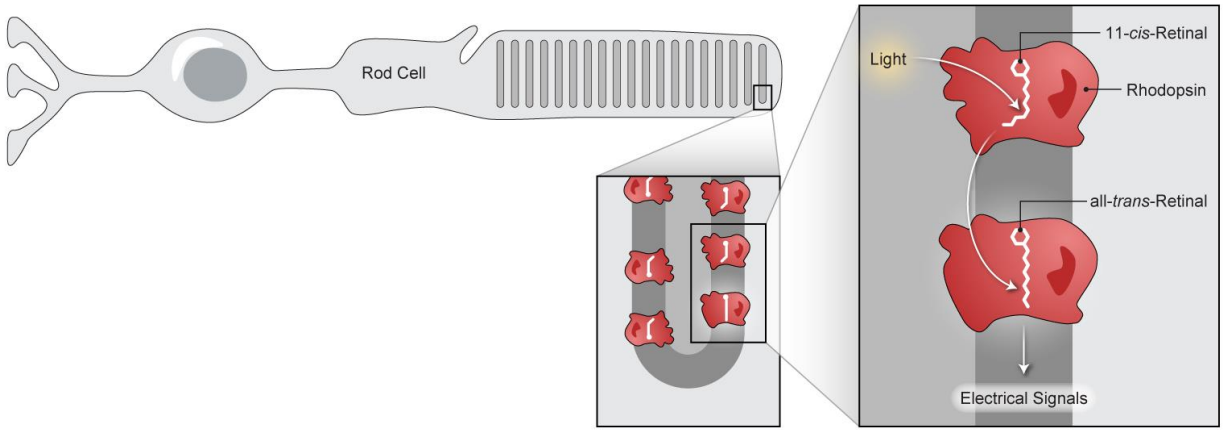


Figure 3. The visual cycle starts when light enters the retina. The rod cells in the retina contain the visual pigment rhodopsin, which is made up of the molecules opsin and 11-*cis*-retinal. When a photon strikes rhodopsin, 11-*cis*-retinal is converted to all-*trans*-retinal. The conversion changes the conformation of rhodopsin, enabling it to initiate a series of chemical reactions that end with electrical signals being transmitted to the brain. The visual cycle requires the RPE65 enzyme to catalyze the conversion of all-*trans*-retinal back to 11-*cis*-retinal. RPE65 is produced in RPE cells and delivered to rod cells. Without it, the cycle is disrupted and rod cells eventually die.

When the RPE65 protein is absent, the rods are less able to respond to light. Over time, the malfunctioning rod cells degenerate and die.

Drs. Bennett and Maguire wondered whether gene therapy could be used to treat LCA by transplanting a functional *RPE65* gene to retinal cells, where it could be expressed and produce functional RPE65 protein. Two things that they needed for a successful gene therapy approach were a suitable vector and a useful animal model.

GENE THERAPY VECTORS

Scientists use viruses as a vector for gene therapy. A vector is a means by which genetic material can be transferred from one organism to another. To ensure their safety and efficacy, researchers remove most of the genes that are part of the virus (such as the genes that the virus needs to make more copies of itself) and replace them with the corrective gene they want to deliver to cells in addition to regulatory sequences that ensure the corrective gene's expression in the target cells.

There are two main classes of viral vectors: those that integrate into the host cell's chromosomes and those that don't. Viral vectors derived from retroviruses are able to integrate into the host cell's DNA. They are typically used to deliver genes inside cells that actively divide. For example, retrovirus-based vectors have been used in gene therapy to deliver corrective genes to bone marrow precursor cells, which then divide and differentiate into different types of immune cells in the body. As the cells divide, they pass on their DNA with the integrated corrective gene to all the daughter cells. One major risk of using integrating vectors is that they integrate randomly in the host cell's chromosomes and can cause mutations at the site of integration.

The second class of viral vectors includes nonintegrating viruses, such as adeno-associated virus (AAV). AAV has become one of the most commonly used viral vectors for delivering genes to cells that are not actively dividing. One major advantage of using AAV is that this virus doesn't typically trigger a harmful immune response in the body. In addition, corrected genes delivered using AAV vectors have been shown to be expressed at high levels in the patient's cells and for many years.

In the film, scientists used a virus vector based on the AAV serotype 2 (AAV2). The AAV2 genome is a linear, single-stranded DNA with about 4,700 base pairs (4.7 kb). Scientists modified the DNA by removing most of the viral genes and inserting the 1.6-kb human *RPE65* cDNA. cDNA (complementary DNA) is DNA synthesized from an RNA template, in this case from the mRNA transcribed from *RPE65*. A cDNA is smaller in size than the gene because it lacks introns. To ensure that the cDNA is expressed, it is attached to promoter and enhancer sequences. Scientists tested many different promoters and enhancers from different species, including ones that are specific to human RPE cells, but found that a cytomegalovirus promoter and chicken beta-actin enhancer resulted in the highest levels of expression in retinal cells (Figure 4).

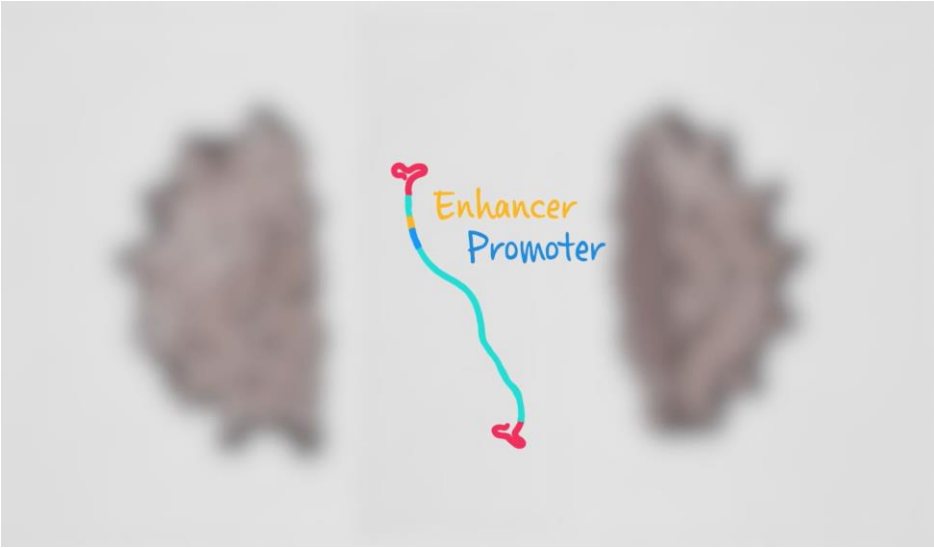


Figure 4. The human *RPE65* cDNA (shown in light blue) replaced most of the genes of the genome of an AAV2 virus (shown in red). Regulatory elements (enhancer and promoter) were added to ensure the cDNA expression inside human cells. (The blurry semicircles on each side are the viral coat.)

To treat LCA, scientists produce large amounts of the AAV2 vector and inject billions of the particles into the patient's retina. The AAV2 viral vector infects retinal cells and delivers its genetic material, including the *RPE65* cDNA. Recall that AAV is a nonintegrating virus, so the transplanted DNA persists primarily as an extrachromosomal element inside cells, which means that it does not become part of one of the cell's chromosomes. Nevertheless, the cell's RNA polymerase recognizes the promoter and transcribes the associated cDNA to produce a messenger RNA, which is then translated by the cell's ribosomes into functional RPE65 protein (Figure 5).

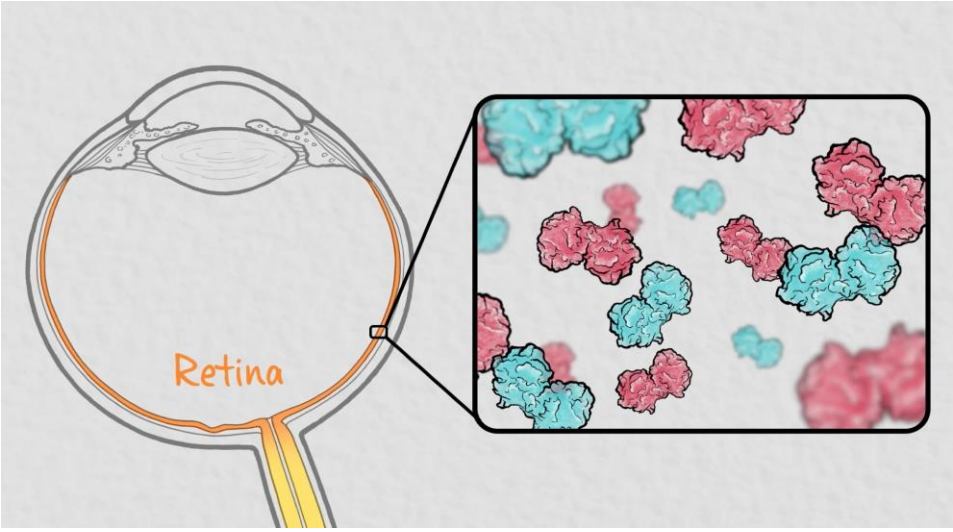


Figure 5. The retinal cells of an individual with LCA produce nonfunctional RPE65 protein (red). Once the corrective gene is introduced via the AAV2 viral vector, the cells should start producing functional RPE65 protein (blue). The hope of this gene therapy treatment is that the functional protein, once expressed in sufficient quantities, will restore the visual cycle.

ANIMAL MODELS

In *Genes as Medicine* we learn that Drs. Bennett, Maguire, and their colleagues first tested their virus-gene delivery method on blind mice. Providing the corrective gene in the mice retinal cells restored their sight. The mice were not ideal animal models for gene therapy treatment, however, because their eyes are so much smaller than those of humans. Briard shepherds, however, not only have similarly sized eyes to those of humans, but they, too, can be affected by LCA (Figure 6).

Figure 6. Briard shepherd puppies

(https://commons.wikimedia.org/wiki/File:Briard_R_01_Puppy.jpg#/media/File:Briard_R_01_Puppy.jpg)

In Briard dogs, a deletion of four base pairs in the *RPE65* gene results in a frameshift mutation and a premature stop codon. The stop codon truncates the RPE65 protein during translation in a way that renders it nonfunctional as an enzyme. Dogs homozygous for the mutation develop vision impairment early in life. Whereas many different mutations in the *RPE65* gene are associated with LCA in humans, all Briard shepherds with LCA have the same mutation, suggesting a founder effect. That is, today's Briard shepherds can be traced back to just a few founding individuals, some of which carried the mutation.

The scientists published their dog trial results in the journal *Nature Genetics* in 2001 and showed that they had successfully restored the dogs' visual function using gene therapy. They continued to follow the dogs and conducted several more experiments with additional dogs, which demonstrated that the therapy was successful at restoring sight for the lifetime of the dogs (over 10 years).

HUMAN TRIALS

After successful experiments in mice, dogs, and even testing the vector in nonhuman primates that did not suffer from blindness, Dr. Bennett and her colleagues began phase 1 human trials in 2007. Phase 1 trials focus primarily on safety and dosage. They are followed by phase 2 trials, which focus not only on safety and dosage, but also on efficacy. Phase 3 trials test for safety, dosage, efficacy, and side effects (Figure 7).

A phase 3 clinical human trial has recently been completed and published with promising results: patients receiving the *RPE65* gene therapy had significant gains in visual acuity compared to a control group. In December 2017, the U.S. Food and Drug Administration (FDA) approved this reagent, now called voretigene neparvovec-rzyl or Luxterna®, as a drug, making it the first gene therapy approved for use to treat a genetic disease.

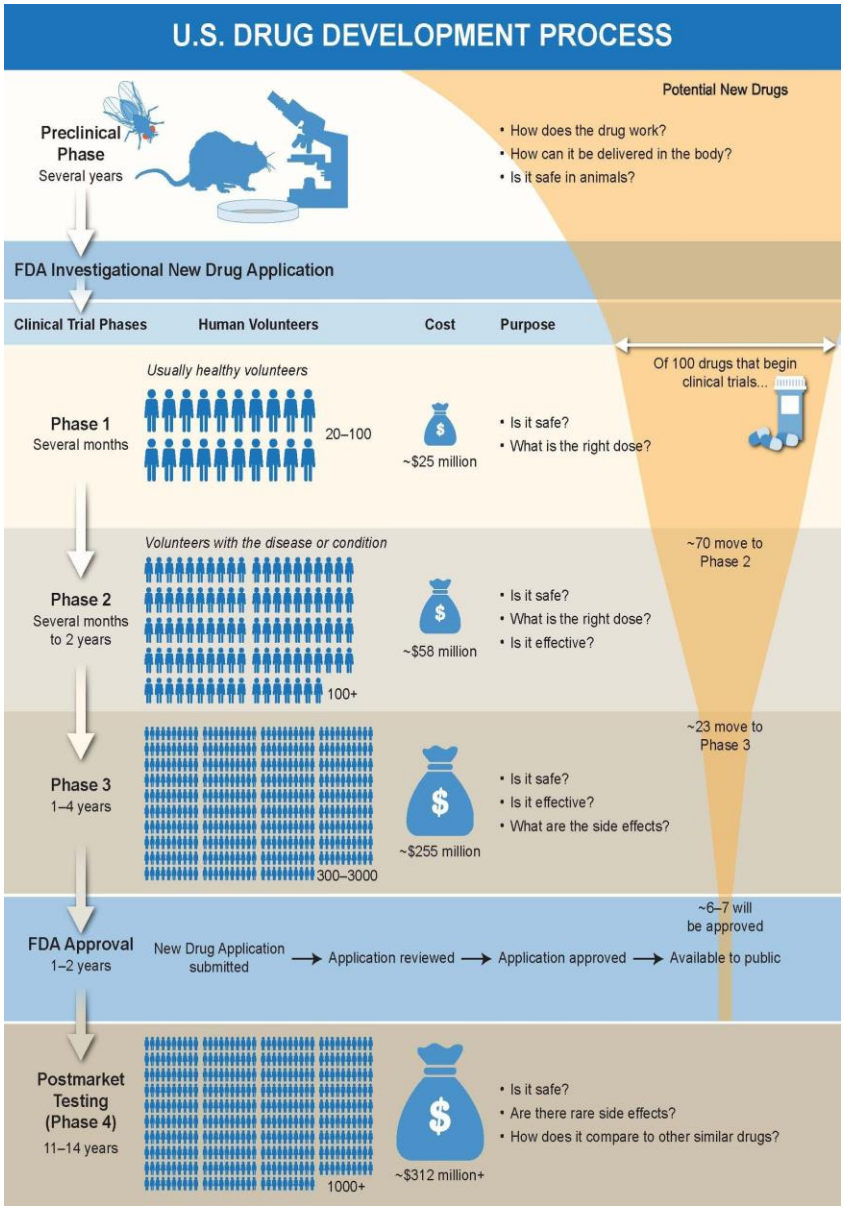


Figure 7. The typical development process for any drug that is approved for use in patients in the United States.

DISCUSSION POINTS

- Remind students that mutations are changes to the DNA. Every individual has variations in their DNA when compared to other individuals. Most mutations are neutral, meaning that they don't have any effect on traits. Mutations that cause disease typically affect proteins with important functions in the body (but not so important that without them an individual could not survive). More information about disease-causing mutations is available in the Click and Learn "Genetic Mutations and Disease" (http://www.hhmi.org/biointeractive/genetic-mutations-and-disease-interactive), including the difference between germline and somatic mutations. For mutations to be inherited from one generation to the next, they need to occur in the germline either as de novo (new) mutations or be inherited from a parent.
- Eyes have several advantages as a target tissue for gene therapy. As mentioned in the film, they are easy to access and observe. In an experiment, one eye can serve as a control, and if the therapy damages one eye, the other one is unaffected. One other important advantage is that the eye is an immune-privileged tissue. This means that the eye, like the brain, triggers fewer immune responses than other tissues. Immune privilege is thought to reflect an evolutionary adaptation to protect vital structures from damage by inflammatory responses directed against pathogens. Ask students to make and defend a claim, based on these characteristics, about which other tissues might be good targets for gene therapy.
- We learn in the film that Jesse Gelsinger died from a strong immune response to the viral vector used in an attempt to treat his liver disease. The vector used in Jesse Gelsinger's gene therapy was an adenovirus (AV), which is derived from the virus frequently responsible for causing the common cold. Researchers now know that AV causes a strong immune response in the body. The vector used by Dr. Bennett is AAV2, which, in its wild-type form, can infect people but does not cause disease. It does not typically activate immune responses that can harm tissues. The virus was tested in animals at much higher doses than would ever be given to humans and did not cause a toxic immune response. Also, the target tissue in the Gelsinger case was the liver, which is not an immune-privileged tissue like the eye is (see earlier note).
- Students may have heard about CRISPR and may wonder how it differs from gene therapy. CRISPR is a tool, derived from bacteria, for directly editing genetic material. CRISPR technologies enable scientists to change the sequence of the DNA inside a cell. They can use them to, for example, introduce mutations in genes to knock out their function, or fix a mutation that causes a disease, or introduce a new gene. Gene therapy, on the other hand, does not edit the cell's own DNA. Instead, it adds a corrective gene to cells. For more information about different genetic technologies used in medicine, visit the HHMI BioInteractive resource "Central Dogma and Genetic Medicine" (http://www.hhmi.org/biointeractive/central-dogma-and-genetic-medicine).
- Ask students how gene therapy differs from other medicines they know about. In the film, Dr. Bennett mentions that gene therapy treats disease "at its root." What that means is that the treatment targets the underlying cause of the disease (in this case a mutation) rather than the symptoms. For example, glasses treat the symptoms of a condition that affects the eyes. Aspirin is a medicine that targets the symptoms (such as a fever) of a disease rather than its cause (such as a virus). Another example of a drug that treats disease at its root is Gleevec, which is treats a form of leukemia. Gleevec disrupts the damaging activity of a mutant protein built from instructions of a mutant gene. A helpful animation can be found here: https://www.hhmi.org/biointeractive/gleevec-inhibits-cancer-causing-kinase-bcr-abl
- Students may ask why Molly's eyesight is not completely restored. Remind them that LCA is a progressive disease that causes photoreceptor cells to die over time. For gene therapy to work, the virus with the corrective gene has to infect target cells and be expressed. Molly was 11 when she underwent gene therapy, and so a significant number of her photoreceptor cells were presumably already dead or severely damaged. Thus, Molly still has some vision loss, though the progression of her disease was halted and she was able to regain a great deal of vision through the expression of the RPE65 protein within her remaining photoreceptor cells. Ask students why *RPE65* gene therapy works best in younger patients.

- Students may wonder what the difference is between an ophthalmologist, an optometrist, and an optician. Dr. Jean Bennett and Dr. Albert Maguire are ophthalmologists: medical doctors (M.D.s) that specialize in diseases of the eyes and can diagnose and treat eye diseases. Some ophthalmologists, like Dr. Maguire, perform eye surgeries. An optometrist is not a medical doctor but a doctor of optometry (O.D.) and a specialist in vision care, which includes diagnosing, treating, and managing vision changes. Opticians specialize in designing and fitting eyeglass lenses and frames as well as contact lenses. Dr. Bennett has both a PhD and an MD. She is trained to do research in the lab as well as to treat patients.
- An interesting discussion could be to have student teams try to list all of the experts/specialists that were involved in some way in the research to find a treatment for LCA. A good but incomplete list derived from a careful viewing of the film might include ophthalmologists, neurologists, veterinarians, virologists, geneticists, and eye surgeons. As an extension, have students research a more complete list and write descriptions of what each expert does.
- Students are familiar with doing experiments in their lab courses and getting results quickly, sometimes during a single class period. Explain that clinical research begins with an idea that first must be shown to work in animal models. If the technique works, a clinical trial can be designed and launched. This process involves first designing the protocol for the study: who is eligible, what tests, dosages, and procedures will be used, how long should the study be conducted, and what data should be recorded. Then an independent committee at the research institution called the Institutional Review Board (IRB) must approve the study. The IRB determines whether the techniques used in the clinical trial are ethical and ensures that the rights of the human participants are protected. Once approved, the investigators must recruit the human test subjects and obtain their informed consent to participate in the trial. Informed consent involves informing participants of all the procedures, risks, and benefits of the trial. An important aspect of clinical trials is that participants are volunteers and are free to remove themselves at any point during the trial. Finally, clinical trials, which involve at least three phases, are expensive and require securing funding and sponsorship from various sources before beginning them. Funding and sponsorship sources include medical institutions, federal agencies, and pharmaceutical companies. In the United States, of every 100 drugs that enter clinical trials, approximately six or seven are eventually made available to the public. The clinical trial process takes many years.
- Ask students whether they think gene therapy would work better for a recessive disease or a dominant disease. In general, gene replacement is more feasible in patients with recessive diseases, which are usually caused by mutations that cause a protein to become nonfunctional (loss-of-function mutation). In contrast, dominant diseases typically involve mutations that cause a protein to gain a new, damaging function (gain-of-function mutations). Gain-of-function mutations require two steps of therapeutic intervention: 1) inactivation or depletion of the dominant allele or protein and 2) introduction and expression of the corrective gene. This two-step approach is more challenging.

STUDENT HANDOUT

We designed the student handout as a learning assessment that probes students' understanding of the key concepts addressed in the film, which can be used before or during the film to assess students' prior knowledge and to guide students as they watch the film. We encourage you to choose the use that best fits your learning objectives and your students' needs. Moreover, because the vocabulary and concepts are complex, we encourage you to modify the handout as needed (e.g., reducing the number of questions, explanations of complicated vocabulary for English learner students).

ANSWERS

1. (Key Concepts E & F) True/False. Soon after a gene associated with childhood blindness was discovered in the 1990s, Drs. Bennett and Maguire were able to offer a gene therapy treatment for children.
   False. It took almost two decades before any treatment for childhood blindness was tried on humans.

2. (Key Concepts E & F) Explain the reasoning or evidence you used to answer question 1.
   After watching the film, students should understand that the process of making a new discovery available to people requires the collaboration of many different areas of science, successful testing on animal models, and clinical trials with human volunteers. These steps often take many years.

3. (Key Concept A) In the film, you met Molly, who has a form of childhood blindness called Leber amaurosis (also called Leber congenital amaurosis, or LCA). Her blindness is caused by
   a. early exposure to intensely bright light.
   b. an eye injury she received at a young age.
   c. mutations in a gene that is necessary for maintaining sight.
   d. a viral disease in her nervous system that moved into her eyes.

4. (Key Concept B) For many genetic diseases, children inherit the disease-causing mutations from their parents. What was the likely inheritance pattern in the case of Molly's form of childhood blindness?
   a. Only females can have Leber amaurosis, so Molly must have inherited the genetic mutation only from her mother.
   b. Molly became blind from new mutations that occurred in her eye as a baby.
   c. Molly ended up with childhood blindness because both of her parents were blind when they were children.
   d. Molly inherited a mutation in the same gene from each of her parents.

5. (Key Concept F) Which statements are reasons that eyes are a good target for clinical trials related to genetic medicine?

   I. Eyes are easy to access.
   II. Eyes are the least important of the five senses.
   III. Eyes in humans are identical to eyes of other species used in animal models.
   IV. One eye can be treated while the other can act as an experimental control.

   a. I and II only
   b. II and III only
   c. III and IV only
   d. I and IV only

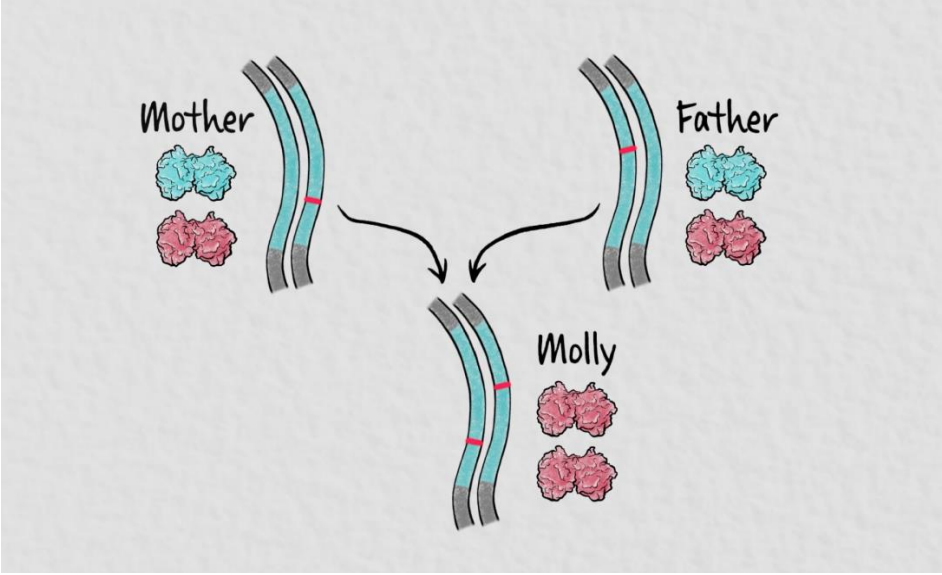
6. (Key Concept D) The gene therapy technique designed by Drs. Bennett and Maguire to cure Molly's blindness involved using a virus as a vector to deliver the corrective gene into one of her eyes. What necessary step must be taken before viruses are used in this way?
   a. Patients must first take flu medicine to help them avoid any flulike symptoms that the virus causes.
   b. The virus's harmful genes and the ones the virus needs to replicate must be removed.
   c. Patients like Molly must first receive a vaccine for the virus so that they don't get the disease that the virus carries.
   d. Doctors must first snip out the defective gene from a patient's photoreceptor cells before using the virus to deliver the corrective gene.

7. (Key Concepts C & D) Explain unique characteristics of viruses that make them useful tools for gene therapy.
   A sufficient answer should describe how viruses are able to move genetic material across cell membranes and how the inserted genetic material can be transcribed into proteins in a patient's cells. Students may also mention that the genome of viruses can be manipulated by scientists to contain copies of genes that will benefit patients.

8. (Key Concept F) As a final test of their technique to cure Leber amaurosis, Drs. Bennett and Maguire needed a large animal model that was similar to humans.
   a. What animal did they choose? ***Drs. Bennett and Maguire chose dogs, specifically Briard shepherds***.
   b. List at least three reasons that this animal was a good model for testing a cure for childhood blindness.
   Students' answers should include three of the following reasons that dogs are useful models:
      - ***Dog eyes are large and about the same size as human eyes.***
      - ***Dogs are visual animals like humans, and testing their ability to see requires little more than watching their behavior.***
      - ***Dog eyes and human eyes have many of the same anatomical features.***
      - ***Just like humans, the Briard shepherd can suffer from Leber amaurosis caused by mutations in the*** RPE65 ***gene, similar to humans.***

9. (Key Concept A) Mutations to the *RPE65* gene can cause Leber amaurosis. Why is it a mistake to call *RPE65* "the Leber amaurosis gene"?
   Calling RPE65 ***"the Leber amaurosis gene" suggests that the function of the gene is to code for a protein that causes the disease. In fact, RPE65 codes for a protein that is crucial for vision. The disease occurs when a person inherits two copies of the gene that each have disease-causing mutations.***

10. (Key Concepts A & B) Sketch and annotate a diagram that shows how Molly ended up inheriting nonfunctional alleles for the gene associated with Leber amaurosis (Molly's form of childhood blindness).



This figure from the film (also Figure 2 in this document) shows an example of a sketch that shows how Molly inherited two mutated alleles of the same gene that produce nonfunctional proteins. A complete answer should be annotated to demonstrate that each of Molly's parents had one working and one mutated allele for the RPE65 ***gene and that Molly inherited a mutated allele from each parent.***

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